

Epigenetic regulation of cholinergic receptor M1 (CHRM1) by histone H3K9me3 impairs Ca²⁺ signaling in Huntington's disease

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Supplementary Methods

Tet-inducible ESET (H3K9 specific histone methyltransferase) cell line

The T-REx™ System (Invitrogen, Carlsbad, CA) was used to generate ESET cell lines. This system utilized two vectors, the pcDNA6/TR vector, a regulatory plasmid that expresses the tetracycline repressor (TetR), and pcDNA5/TO that contains a CMV promoter driving the expression of the gene of interest under the control of Tet-operator sequences. Flag-ESET were subcloned into the pcDNA5/TO vector from pcDNA-Flag-ESET construct, in which full length of ESET is cloned to a CMV-driven vector (Clontech, Mountain View, CA). pcDNA5/TO-Flag-ESET was linearized and transfected into Q7 striatal cell clone over expressing pcDNA6/TR. The ESET cell clones was selected by hygromycin. For the induction of ESET, 3-10 μ M of doxycycline was treated into culture medium.

Supplementary Table 1 Gene ontology Biological Processes (GOBPs) enriched ($P < 0.05$) by the genes with low H3K9 promoter occupancy and high RNA expression (up-regulated genes). **a** Three groups of GOBPs used to reconstruct the network model (Fig. 2e). **b** A list of all the GOBP terms with p -value < 0.05 .

a

	GOBP Terms		Count	P-value	Genes
synaptic transmission	GO:0019226	transmission of nerve impulse	15	2.26E-03	EGR3, NRXN2, UGT8A, SNCA, STX1B, CD9, HRH1, CHRM1, ABAT, UNC13C, SHC3, NCAN, UNC13B, CACNA1A, TES
	GO:0007268	synaptic transmission	15	2.28E-03	EGR3, NRXN2, SNCA, STX1B, HRH1, CHRM1, ABAT, UNC13C, SHC3, NCAN, UNC13B, CACNA1A, TES, CHRND, LAMA2
axon guidance	GO:0007409	axonogenesis	10	2.57E-02	EPHA4, CXCR4, EFNB1, LHX2, KIF5C, NTNG1, ETV1, CNP, GLI2, SLIT2
	GO:0031175	neuron projection development	13	1.12E-02	EFNB1, KIF5C, NTNG1, CNP, GLI2, SLIT2, EPHA4, LINGO1, PPP1R9A, CXCR4, LHX2, ETV1, CACNA1A
	GO:0007411	axon guidance	8	1.34E-02	EPHA4, CXCR4, EFNB1, LHX2, KIF5C, ETV1, GLI2, SLIT2
actin cytoskeleton	GO:0016477	cell migration	16	1.46E-03	SCHIP1, PDGFB, PLXNA2, EFNB1, PODXL, ITGA1, ASTN1, ITGA3, ITGA4, CX3CL1, SYNE2, CXCR4, CTGF, LRP8, NR2F2, TES
	GO:0051056	regulation of small GTPase mediated signal transduction	12	3.50E-02	FGD2, RASGRF2, ARHGEF6, PREX2, IQGAP2, RASGEF1A, PSD2, ARAP3, RASA3, TBC1D2, ALS2CL, FGD4
	GO:0007229	integrin-mediated signaling pathway	6	4.66E-02	ITGAL, CTGF, ITGA1, ADAM33, ITGA3, ITGA4

b

	GOBP Terms	Count	P-value	Genes
GO:0007155	cell adhesion	34	9.12E-06	ITGAL, PCDHA8, CADM1, PCDHA3, CLSTN3, NINJ2, ASTN1, NEDD9, CX3CL1, MEGF10, CD9, CTGF, TEK, KLRA7, ESAM, AMICA1, COL8A1, CDH26, COL11A1, HAPLN4, CDHR1, ITGA1, EFS, ITGA3, PTPRU, ITGA4, PCDH19, GPR98, LAMA2, DDR1, CDH13, AMIGO2, WNT7B, NCAN
GO:0022610	biological adhesion	34	9.53E-06	ITGAL, PCDHA8, CADM1, PCDHA3, CLSTN3, NINJ2, ASTN1, NEDD9, CX3CL1, MEGF10, CD9, CTGF, TEK, KLRA7, ESAM, AMICA1, COL8A1, CDH26, COL11A1, HAPLN4, CDHR1, ITGA1, EFS, ITGA3, PTPRU, ITGA4, PCDH19, GPR98, LAMA2, DDR1, CDH13, AMIGO2, WNT7B, NCAN
GO:0016337	cell-cell adhesion	20	1.21E-05	ITGAL, PCDHA8, CADM1, PCDHA3, CLSTN3, CDHR1, ASTN1, PTPRU, ITGA4, PCDH19, GPR98, CDH13, AMIGO2, WNT7B, CTGF, TEK, ESAM, NCAN, CDH26, COL11A1
GO:0043583	ear development	13	1.50E-05	MAFB, OTX1, TMIE, SOX2, EDN1, JAG2, JAG1, GPR98, PTPRQ, DDR1, EYA1, CLIC5, COL11A1
GO:0006928	cell motion	25	2.99E-05	SCHIP1, PDGFB, PLXNA2, ASTN1, 1810024B03RIK, CX3CL1, GLI2, CD9, CXCR4, CTGF, LHX2, ETV1, NR2F2, TES, SMOK4A, PODXL, EFNB1, KIF5C, ITGA1, ITGA3, ITGA4, SLIT2, EPHA4, SYNE2, LRP8
GO:0007267	cell-cell signaling	21	6.67E-05	EGR3, NRXN2, LTBP4, SOX2, SNCA, FZD1, ILDR2, GLI2, STX1B, HRH1, WNT7B, WNT4, WNT3, CHRM1, ABAT, NCAN, SHC3, UNC13C, UNC13B, CACNA1A, TES
GO:0048839	inner ear development	11	8.78E-05	EYA1, MAFB, CLIC5, OTX1, TMIE, SOX2, JAG2, JAG1, COL11A1, GPR98, PTPRQ
GO:0060429	epithelium development	18	6.97E-04	BMP2, JAG2, FZD1, HOXB13, SPRR2F, JAG1, SPRR2E, GLI2, SPRR2K, ZIC2, DDR1, WNT4, EYA1, EVPL, IRF6, PPL, POU2F3, SPRR2B, CAR2
GO:0042471	ear morphogenesis	9	7.48E-04	EYA1, MAFB, CLIC5, OTX1, TMIE, EDN1, SOX2, COL11A1, PTPRQ
GO:0051674	localization of cell	18	1.16E-03	SCHIP1, SMOK4A, PDGFB, PLXNA2, EFNB1, PODXL, 1810024B03RIK, ITGA1, ASTN1, ITGA3, CX3CL1, ITGA4, SYNE2, CXCR4, CTGF, LRP8, NR2F2, TES
GO:0007389	pattern specification process	18	1.16E-03	OEEP, MAFB, EFNB1, OTX1, EDN1, GRHL3, ZBTB16, GLI2, CTNNBIP1, HOXC6, EYA1, WNT3, CXCR4, LHX2, HHIP, NR2F2, CHR1, MLLT3
GO:0048870	cell motility	18	1.16E-03	SCHIP1, SMOK4A, PDGFB, PLXNA2, EFNB1, PODXL,

				1810024B03RIK, ITGA1, ASTN1, ITGA3, CX3CL1, ITGA4, SYNE2, CXCR4, CTGF, LRP8, NR2F2, TES
GO:0030216	keratinocyte differentiation	7	1.40E-03	EVPL, IRF6, PPL, POU2F3, SPRR2B, SPRR2F, SPRR2E, SPRR2K
GO:0016477	cell migration	16	1.46E-03	SCHIP1, PDGFB, PLXNA2, EFNB1, PODXL, ITGA1, ASTN1, ITGA3, ITGA4, CX3CL1, SYNE2, CXCR4, CTGF, LRP8, NR2F2, TES
GO:0042472	inner ear morphogenesis	8	1.66E-03	EYA1, MAFB, CLIC5, OTX1, TMIE, SOX2, COL11A1, PTPRQ
GO:0009266	response to temperature stimulus	7	1.92E-03	ADRB3, ADRB1, STAC, TRPV2, ST8SIA1, HSPA1A, SCARA5
GO:0009913	epidermal cell differentiation	7	1.92E-03	EVPL, IRF6, PPL, POU2F3, SPRR2B, SPRR2F, SPRR2E, SPRR2K
GO:0019226	transmission of nerve impulse	15	2.26E-03	EGR3, NRXN2, UGT8A, SNCA, STX1B, CD9, HRH1, CHRM1, ABAT, UNC13C, SHC3, NCAN, UNC13B, CACNA1A, TES
GO:0009628	response to abiotic stimulus	16	2.26E-03	SLC12A2, TRPV2, SOX2, ST8SIA1, MGP, HSPA1A, GPR98, ADRB3, EYA1, ADRB1, STAC, PDE1B, ETV1, SCARA5, OPN3, RHO
GO:0007268	synaptic transmission	15	2.28E-03	EGR3, NRXN2, SNCA, STX1B, HRH1, CHRM1, ABAT, UNC13C, SHC3, NCAN, UNC13B, CACNA1A, TES, CHRND, LAMA2
GO:0007156	homophilic cell adhesion	10	3.30E-03	CDH13, AMIGO2, PCDHA8, CADM1, PCDHA3, CLSTN3, CDHR1, ESAM, CDH26, PCDH19
GO:0042127	regulation of cell proliferation	26	3.36E-03	ITGAL, PDGFB, EDN1, SOX2, BTC, ST8SIA1, JAG2, ZBTB16, GLI2, IL11, CD9, TEK, ADAM33, TES, BMP2, TESC, KLB, FLT3, CRIP2, EFNB1, RASIP1, CD40, DDR1, IRF6, ID4, ALOX8
GO:0000904	cell morphogenesis involved in differentiation	14	3.49E-03	BMP2, EFNB1, KIF5C, NTNG1, CNP, GLI2, SLIT2, EPHA4, WNT4, CXCR4, LHX2, CLIC5, ETV1, CACNA1A
GO:0030030	cell projection organization	18	3.93E-03	PDGFB, EFNB1, KIF5C, NTNG1, CNP, GLI2, GPR98, SLIT2, EPHA4, LINGO1, PPP1R9A, CXCR4, CLIC5, LHX2, ETV1, CACNA1A, TUBB4, FGD4
GO:0048568	embryonic organ development	15	4.04E-03	MAFB, OTX1, TMIE, SOX2, EDN1, ITGA4, GLI2, TCF7L2, CITED1, PTPRQ, WNT7B, EYA1, CLIC5, TCFEB, COL11A1
GO:0032989	cellular component morphogenesis	19	4.54E-03	BMP2, OOEP, EFNB1, KIF5C, NTNG1, CNP, GLI2, SLIT2, ACTG1, TNNT2, EPHA4, CD9, WNT4, SYNE2, CXCR4, CLIC5, LHX2, ETV1, CACNA1A
GO:0030855	epithelial cell differentiation	10	4.60E-03	WNT4, EVPL, IRF6, PPL, POU2F3, SPRR2B, FZD1, HOXB13, SPRR2F, SPRR2E, SPRR2K
GO:0031424	keratinization	5	5.49E-03	EVPL, PPL, SPRR2B, SPRR2F, SPRR2E, SPRR2K
GO:0048598	embryonic morphogenesis	19	5.73E-03	MAFB, OTX1, TMIE, SOX2, EDN1, JAG2, ZBTB16, ITGA4, GLI2, TCF7L2, PTPRQ, ZIC2, WNT7B, WNT4, EYA1, WNT3, CLIC5, CHRDL, COL11A1
GO:0007423	sensory organ development	15	7.06E-03	MAFB, OTX1, TMIE, SOX2, EDN1, JAG2, JAG1, NHS, GPR98, PTPRQ, DDR1, EYA1, CLIC5, COL8A1, COL11A1
GO:0048667	cell morphogenesis involved in neuron differentiation	12	7.72E-03	EPHA4, CXCR4, EFNB1, CLIC5, LHX2, KIF5C, NTNG1, ETV1, CNP, GLI2, CACNA1A, SLIT2
GO:0048562	embryonic organ morphogenesis	11	9.04E-03	EYA1, MAFB, CLIC5, OTX1, TMIE, EDN1, SOX2, GLI2, COL11A1, TCF7L2, PTPRQ
GO:0048729	tissue morphogenesis	14	9.04E-03	BMP2, JAG2, HOXB13, JAG1, GLI2, ZIC2, TNNT2, DDR1, EYA1, WNT4, WNT3, CHRDL, COL11A1, CAR2
GO:0003002	regionalization	13	9.79E-03	MAFB, OTX1, EDN1, ZBTB16, GLI2, HOXC6, CTNNBIP1, WNT3, LHX2, HHIP, NR2F2, CHRDL, MLLT3
GO:0043627	response to estrogen stimulus	5	9.92E-03	SPRR2B, SPRR2F, CFTR, SPRR2E, TIMP3, TES
GO:0006865	amino acid transport	7	9.96E-03	SLC7A3, SLC3A2, SLC38A1, SLC7A5, SLC43A1, CACNA1A, SLC7A11
GO:0030155	regulation of cell adhesion	8	1.09E-02	LAMA2, ITGAL, DDR1, TESC, PODXL, B4GALNT2, JAG2, COL8A1
GO:0031175	neuron projection development	13	1.12E-02	EFNB1, KIF5C, NTNG1, CNP, GLI2, SLIT2, EPHA4, LINGO1, PPP1R9A, CXCR4, LHX2, ETV1, CACNA1A
GO:0007411	axon guidance	8	1.34E-02	EPHA4, CXCR4, EFNB1, LHX2, KIF5C, ETV1, GLI2, SLIT2
GO:0046942	carboxylic acid transport	8	1.42E-02	SLC7A3, PLIN2, SLC3A2, SLC38A1, SLC7A5, SLC43A1, CACNA1A, SLC7A11
GO:0000902	cell morphogenesis	16	1.46E-02	BMP2, OOEP, EFNB1, KIF5C, NTNG1, CNP, GLI2, SLIT2, EPHA4, WNT4, SYNE2, CXCR4, LHX2, CLIC5, ETV1, CACNA1A
GO:0015849	organic acid transport	8	1.49E-02	SLC7A3, PLIN2, SLC3A2, SLC38A1, SLC7A5, SLC43A1, CACNA1A, SLC7A11
GO:0001763	morphogenesis of a branching structure	9	1.58E-02	DDR1, WNT4, BMP2, EYA1, PDGFB, CXCR4, EDN1, HOXB13, GLI2
GO:0008544	epidermis development	9	1.58E-02	EVPL, IRF6, PPL, POU2F3, SPRR2B, SPRR2F, GRHL3, SPRR2E, SPRR2K, EDARADD
GO:0030182	neuron differentiation	19	1.58E-02	EFNB1, KIF5C, SOX2, NTNG1, JAG2, CNP, JAG1, GLI2, GPR98, SLIT2, EPHA4, LINGO1, PPP1R9A, CXCR4, CLIC5, LHX2, ETV1, ID4, CACNA1A
GO:0048812	neuron projection	11	1.61E-02	EPHA4, CXCR4, EFNB1, LHX2, KIF5C, NTNG1, ETV1, CNP, GLI2,

	morphogenesis			CACNA1A, SLIT2
GO:0001501	skeletal system development	15	1.64E-02	SCHIP1, BMP2, EDN1, JAG2, MGP, ZBTB16, GLI2, HOXC6, ALOX15, EYA1, CTGF, MN1, CHRDL, COL11A1, BMP8B
GO:0009612	response to mechanical stimulus	5	1.77E-02	SLC12A2, SOX2, MGP, ETV1, GPR98
GO:0007610	behavior	19	1.81E-02	SNCA, ASTN1, ITGA1, ITGA3, CNP, CX3CL1, SLIT2, CCL17, EPHA4, PLCL1, PTGDS, CCL20, PDE1B, PPP1R1B, PRKAR1B, ABAT, ETV1, SHC3, CACNA1A
GO:0048666	neuron development	15	1.98E-02	EFNB1, KIF5C, NTNG1, CNP, GLI2, GPR98, SLIT2, EPHA4, LINGO1, PPP1R9A, CXCR4, LHX2, CLIC5, ETV1, CACNA1A
GO:0003006	reproductive developmental process	14	1.99E-02	PLAG1, TESC, SOX2, HOXB13, ZBTB16, GLI2, TCF7L2, DMRTA1, INSR, YBX2, HOOK1, WNT4, CXCR4, BIK
GO:0035295	tube development	14	1.99E-02	BMP2, EDN1, MGP, CFTR, GLI2, ZIC2, DDR1, EYA1, WNT4, CXCR4, CTGF, WWP1, HHIP, TES
GO:0045596	negative regulation of cell differentiation	11	1.99E-02	LINGO1, CCND1, WNT4, FLT3, SOX2, ID4, JAG1, ZBTB16, GLI2, TCF7L2, CHRDL
GO:0001503	ossification	8	1.99E-02	ALOX15, BMP2, CTGF, MGP, GLI2, MN1, CHRDL, BMP8B
GO:0050910	detection of mechanical stimulus involved in sensory perception of sound	3	2.12E-02	SLC12A2, SOX2, GPR98
GO:0007398	ectoderm development	9	2.21E-02	EVPL, IRF6, PPL, POU2F3, SPRR2B, SPRR2F, GRHL3, SPRR2E, SPRR2K, EDARADD
GO:0007010	cytoskeleton organization	16	2.26E-02	TPPP3, PDGFB, ELN, CNP, FHDC1, DAAM1, TNNT2, ACTG1, EPB4.1L2, HOOK1, PPP1R9A, PFN4, TAGLN, FHOD3, EPB4.9, TES
GO:0035023	regulation of Rho protein signal transduction	7	2.38E-02	FGD2, RASGRF2, ARHGEF6, PREX2, ARAP3, ALS2CL, FGD4
GO:0040012	regulation of locomotion	8	2.39E-02	LAMA2, PDGFB, CXCR4, CHRM1, DLL4, SNCA, TEK, ARAP3
GO:0007409	axonogenesis	10	2.57E-02	EPHA4, CXCR4, EFNB1, LHX2, KIF5C, NTNG1, ETV1, CNP, GLI2, SLIT2
GO:0030036	actin cytoskeleton organization	10	2.76E-02	EPB4.1L2, TNNT2, ACTG1, PPP1R9A, PDGFB, PFN4, ELN, FHOD3, FHDC1, DAAM1
GO:0006470	protein amino acid dephosphorylation	8	2.83E-02	PTPRK, DUSP4, DUSP18, EYA1, DUSP15, PTPRU, DUSP6, PTPRQ
GO:0045667	regulation of osteoblast differentiation	4	2.86E-02	WNT7B, BMP2, SOX2, CHRDL
GO:0040036	regulation of fibroblast growth factor receptor signaling pathway	3	3.14E-02	WNT4, PDGFB, HHIP
GO:0007379	segment specification	3	3.14E-02	MAFB, GLI2, MLLT3
GO:0034605	cellular response to heat	3	3.14E-02	STAC, ST8SIA1, SCARA5
GO:0042311	vasodilation	4	3.16E-02	ADRB3, ADRB1, CFTR, TES
GO:0015837	amine transport	7	3.18E-02	SLC7A3, SLC3A2, SLC38A1, SLC7A5, SLC43A1, CACNA1A, SLC7A11
GO:0048754	branching morphogenesis of a tube	7	3.33E-02	DDR1, WNT4, BMP2, EYA1, CXCR4, EDN1, GLI2
GO:0060348	bone development	8	3.33E-02	ALOX15, BMP2, CTGF, MGP, GLI2, MN1, CHRDL, BMP8B
GO:0008284	positive regulation of cell proliferation	14	3.35E-02	ITGAL, PDGFB, KLB, CRIP2, EFNB1, SOX2, EDN1, BTC, ST8SIA1, RASIP1, CD40, GLI2, IL11, ID4
GO:0009968	negative regulation of signal transduction	10	3.36E-02	CTNBP1, RGS20, CCND1, WNT4, SOX2, ZFYVE28, HHIP, ARAP3, PHLDA3, CHRDL
GO:0050880	regulation of blood vessel size	5	3.49E-02	ADRB3, ADRB1, EDN1, CFTR, TES
GO:0035150	regulation of tube size	5	3.49E-02	ADRB3, ADRB1, EDN1, CFTR, TES
GO:0051056	regulation of small GTPase mediated signal transduction	12	3.50E-02	FGD2, RASGRF2, ARHGEF6, PREX2, IQGAP2, RASGEF1A, PSD2, ARAP3, RASA3, TBC1D2, ALS2CL, FGD4
GO:0001655	urogenital system development	9	3.58E-02	PLAG1, SCHIP1, WNT4, BMP2, EYA1, HOXB13, ZBTB16, GLI2, SLIT2
GO:0002009	morphogenesis of an epithelium	10	3.58E-02	DDR1, WNT4, BMP2, EYA1, JAG2, HOXB13, JAG1, GLI2, CAR2, ZIC2
GO:0048858	cell projection morphogenesis	11	3.72E-02	EPHA4, CXCR4, EFNB1, LHX2, KIF5C, NTNG1, ETV1, CNP, GLI2, CACNA1A, SLIT2
GO:0030029	actin filament-based process	10	3.92E-02	EPB4.1L2, TNNT2, ACTG1, PPP1R9A, PDGFB, PFN4, ELN, FHOD3, FHDC1, DAAM1
GO:0003018	vascular process in circulatory system	5	3.97E-02	ADRB3, ADRB1, EDN1, CFTR, TES
GO:0050801	ion homeostasis	14	4.13E-02	TESC, ATP4A, UGT8A, SLC9A2, PRND, EDN1, SNCA, TTC7, RHAG, CKB, CD9, CHRND, SCARA5, CACNA1A
GO:0060113	inner ear receptor cell differentiation	4	4.15E-02	CLIC5, JAG2, JAG1, GPR98
GO:0060541	respiratory system	8	4.18E-02	CTGF, WWP1, SOX2, MGP, CFTR, HHIP, GLI2, TES

	development			
GO:0015804	neutral amino acid transport	3	4.30E-02	SLC3A2, SLC38A1, SLC43A1
GO:0009582	detection of abiotic stimulus	5	4.49E-02	SLC12A2, SOX2, OPN3, GPR98, RHO
GO:0055082	cellular chemical homeostasis	13	4.59E-02	TESC, FOXA3, UGT8A, PRND, EDN1, SNCA, TTC7, RHAG, CKB, CD9, CHRND, SCARA5, CACNA1A
GO:0007229	integrin-mediated signaling pathway	6	4.66E-02	ITGAL, CTGF, ITGA1, ADAM33, ITGA3, ITGA4
GO:0030278	regulation of ossification	5	4.76E-02	WNT7B, BMP2, SOX2, MGP, CHR1
GO:0009408	response to heat	4	4.88E-02	STAC, ST8SIA1, HSPA1A, SCARA5
GO:0032990	cell part morphogenesis	11	4.89E-02	EPHA4, CXCR4, EFN1, LHX2, KIF5C, NTNG1, ETV1, CNP, GLI2, CACNA1A, SLIT2
GO:0045685	regulation of glial cell differentiation	3	4.93E-02	LINGO1, BMP2, ID4
GO:0014013	regulation of gliogenesis	3	4.93E-02	LINGO1, BMP2, ID4

Supplementary Table 2 Gene ontology Biological Processes (GOBPs) enriched ($P < 0.05$) by the genes with low H3K9 promoter occupancy and high RNA expression (up-regulated genes).

GOBP Terms	Count	P-value	Genes
GO:0048010	6	6.8.E-06	IGSF10, VEGFC, PDGFRA, PDGFRB, FOXC1, KDR
GO:0007155	28	5.5.E-05	ATP1B2, ZAN, PCDHB14, PCDHB11, SCARF2, LAMB3, WISP2, PVRL2, VNN1, THBS2, DPT, FN1, PCDHB9, TNXB, CLCA5, SDK2, F8, CELSR3, PCDHB20, CLDN10A, KITL, CASS4, STAB1, CD33, PKP3, COL24A1, CHL1, AOC3
GO:0022610	28	5.7.E-05	ATP1B2, ZAN, PCDHB14, PCDHB11, SCARF2, LAMB3, WISP2, PVRL2, VNN1, THBS2, DPT, FN1, PCDHB9, TNXB, CLCA5, SDK2, F8, CELSR3, PCDHB20, CLDN10A, KITL, CASS4, STAB1, CD33, PKP3, COL24A1, CHL1, AOC3
GO:0006955	24	1.6.E-04	IL1R1, CXCL3, RAET1B, RAET1A, H60B, RNF125, LIF, RAET1C, RAET1E, VNN1, MX1, MPA2L, MX2, GBP6, GBP5, C4B, GBP9, MALT1, TINAGL1, PSMB8, CBLB, TNFSF10, H2-T9, IRF7, C1RL, H2-T22, TGTP1, TGTP2, GBP4
GO:0006468	26	2.2.E-03	WNT5A, PRKCZ, LPAR3, TRIB2, IGSF10, LIF, GM711, PKN3, SPEG, MAP3K1, EGF, MAP2K6, DCLK1, GUCY2G, SGK1, TRPM6, ALPK2, PHKG1, PRKCH, KDR, NTRK3, PLCE1, PDGFRA, PDGFRB, TGFRB3, CDK20
GO:0030198	8	5.4.E-03	TNXB, SERPINB5, SMOC1, OLFML2B, PDGFRA, FOXC1, VWA1, DPT
GO:0007169	11	7.5.E-03	IGSF10, NTRK3, TXNIP, VEGFC, DOK2, PDGFRA, PDGFRB, FOXC1, EGF, STAT3, KDR
GO:0043405	7	8.5.E-03	PLCE1, TNXB, MAP3K1, LPAR3, KITL, MAP2K6, TRIB2
GO:0042325	14	8.6.E-03	TNXB, SPHK1, LPAR3, KITL, TRIB2, CDKN1C, LIF, PLCE1, MAP3K1, IKBK, PDGFRB, FAM129A, EGF, MAP2K6
GO:0016310	26	9.4.E-03	WNT5A, PRKCZ, LPAR3, TRIB2, IGSF10, LIF, GM711, PKN3, SPEG, MAP3K1, EGF, MAP2K6, DCLK1, GUCY2G, SGK1, TRPM6, ALPK2, PHKG1, PRKCH, KDR, NTRK3, PLCE1, PDGFRA, PDGFRB, TGFRB3, CDK20
GO:0000165	8	1.0.E-02	WNT5A, DOK2, PLCE1, MAP3K1, LPAR3, ITPKB, EGF, MAP2K6
GO:0007243	12	1.1.E-02	LIF, WNT5A, DOK2, PLCE1, MAP3K1, IKBK, LPAR3, ITPKB, MALT1, EGF, STAT3, MAP2K6
GO:0051174	14	1.1.E-02	TNXB, SPHK1, LPAR3, KITL, TRIB2, CDKN1C, LIF, PLCE1, MAP3K1, IKBK, PDGFRB, FAM129A, EGF, MAP2K6
GO:0019220	14	1.1.E-02	TNXB, SPHK1, LPAR3, KITL, TRIB2, CDKN1C, LIF, PLCE1, MAP3K1, IKBK, PDGFRB, FAM129A, EGF, MAP2K6
GO:0006639	5	1.2.E-02	APOB48R, APOB, TNXB, ANG, APOC1
GO:0007167	13	1.3.E-02	TXNIP, STAT3, KDR, NTRK3, IGSF10, VEGFC, DOK2, MAP3K1, PDGFRA, PDGFRB, TGFRB3, FOXC1, EGF
GO:0006662	5	1.5.E-02	APOB48R, APOB, TNXB, ANG, APOC1
GO:0006638	5	1.5.E-02	APOB48R, APOB, TNXB, ANG, APOC1
GO:0006796	29	1.6.E-02	WNT5A, PRKCZ, CDC14B, PTPN22, LPAR3, TRIB2, IGSF10, LIF, GM711, PKN3, SPEG, MAP3K1, EGF, DCLK1, MAP2K6, GUCY2G, TRPM6, SGK1, ALPK2, PHKG1, PRKCH, KDR, NTRK3, PLCE1, PPM1K, PDGFRA, PDGFRB, TGFRB3, CDK20
GO:0006793	29	1.6.E-02	WNT5A, PRKCZ, CDC14B, PTPN22, LPAR3, TRIB2, IGSF10, LIF, GM711, PKN3, SPEG, MAP3K1, EGF, DCLK1, MAP2K6, GUCY2G, TRPM6, SGK1, ALPK2, PHKG1, PRKCH, KDR, NTRK3, PLCE1, PPM1K, PDGFRA, PDGFRB, TGFRB3, CDK20
GO:0045860	8	1.6.E-02	PLCE1, MAP3K1, SPHK1, IKBK, LPAR3, EGF, KITL, MAP2K6
GO:0045859	10	1.7.E-02	PLCE1, TNXB, MAP3K1, SPHK1, IKBK, LPAR3, EGF, KITL, MAP2K6, TRIB2
GO:0018904	5	1.8.E-02	APOB48R, APOB, TNXB, ANG, APOC1
GO:0033674	8	2.0.E-02	PLCE1, MAP3K1, SPHK1, IKBK, LPAR3, EGF, KITL, MAP2K6
GO:0043549	10	2.0.E-02	PLCE1, TNXB, MAP3K1, SPHK1, IKBK, LPAR3, EGF, KITL, MAP2K6, TRIB2
GO:0009615	6	2.2.E-02	ISG15, ZC3HAV1, IRF7, TGTP1, TGTP2, MX1, MX2

GO:0051347	positive regulation of transferase activity	8	2.4.E-02	PLCE1, MAP3K1, SPHK1, IKBK, LPAR3, EGF, KITL, MAP2K6
GO:0051338	regulation of transferase activity	10	2.5.E-02	PLCE1, TNXB, MAP3K1, SPHK1, IKBK, LPAR3, EGF, KITL, MAP2K6, TRIB2
GO:0045087	innate immune response	7	2.5.E-02	IL1R1, C4B, C1RL, VNN1, MALT1, MX1, MX2
GO:0006811	ion transport	24	2.7.E-02	GABRE, TRPM6, GLRB, SCN3A, ATP1B2, CLCA5, SCN2A1, SLC12A5, TRPV3, SLC24A6, KCNIP3, CNNM1, SLC16A3, SLC1A3, SLC41A3, SLC39A8, CHRNA4, ABCC4, ANO5, HEPH, SCN8A, SLC30A3, SLC4A4, STEAP2
GO:0002526	acute inflammatory response	6	2.8.E-02	C4B, F8, C1RL, VNN1, STAT3, FN1
GO:0016337	cell-cell adhesion	11	2.8.E-02	PCDHB9, TNXB, PVRL2, CELSR3, PCDHB14, VNN1, CLDN10A, PCDHB11, PCDHB20, CHL1, SCARF2
GO:0044093	positive regulation of molecular function	13	2.9.E-02	ADCY7, SPHK1, LPAR3, MALT1, KITL, PLCE1, ANG, MAP3K1, IKBK, TGFBR3, CALCRL, EGF, MAP2K6
GO:0009611	response to wounding	14	3.2.E-02	F2RL3, NFKBID, C4B, CXCL3, SPHK1, HPS1, F8, STAT3, SLC1A3, STAB1, MAP3K1, C1RL, VNN1, FN1
GO:0001568	blood vessel development	11	3.4.E-02	VEGFC, MYO18B, APOB, ANG, SPHK1, MMP19, TGFBR3, FOXC1, MMP2, VASH1, KDR
GO:0043406	positive regulation of MAP kinase activity	5	3.5.E-02	PLCE1, MAP3K1, LPAR3, KITL, MAP2K6
GO:0007156	homophilic cell adhesion	7	3.7.E-02	PCDHB9, PVRL2, CELSR3, PCDHB14, PCDHB11, PCDHB20, CHL1
GO:0043062	extracellular structure organization	8	3.8.E-02	TNXB, SERPINB5, SMOC1, OLFML2B, PDGFRA, FOXC1, VWA1, DPT
GO:0001944	vasculature development	11	3.9.E-02	VEGFC, MYO18B, APOB, ANG, SPHK1, MMP19, TGFBR3, FOXC1, MMP2, VASH1, KDR
GO:0006641	triglyceride metabolic process	4	4.0.E-02	APOB48R, APOB, TNXB, APOC1
GO:0006869	lipid transport	7	4.0.E-02	APOB48R, APOB, APOC1, APOL6, APOM, APOL7B, ABCG1
GO:0001932	regulation of protein amino acid phosphorylation	7	4.3.E-02	LIF, TNXB, MAP3K1, PDGFRB, FAM129A, EGF, KITL
GO:0006814	sodium ion transport	7	4.9.E-02	SCN3A, ATP1B2, SLC12A5, SCN2A1, SLC24A6, SCN8A, SLC4A4
GO:0006954	inflammatory response	10	4.9.E-02	NFKBID, C4B, STAB1, CXCL3, SPHK1, F8, C1RL, VNN1, STAT3, FN1
GO:0005996	monosaccharide metabolic process	9	4.9.E-02	PCX, UGT1A9, H6PD, PHKG1, UGT1A6B, PFKFB3, UGT1A6A, CMAH, RBKS, DHTKD1, CPT1A
GO:0043085	positive regulation of catalytic activity	11	5.0.E-02	PLCE1, ADCY7, ANG, MAP3K1, SPHK1, IKBK, LPAR3, CALCRL, EGF, KITL, MAP2K6

Supplementary Table 3. Human brain samples that are used in the immunohistochemistry and qRT-PCR. The grades of HD samples were between 2-3.

Control	Age	Gender
1	87	F
2	61	M
3	81	M
4	81	M
5	87	F
6	67	M
7	86	M
Huntington's disease	Age	Gender
1	49	M
2	72	M
3	55	M
4	80	M
5	48	M

Supplementary Table 4 Primer sets that are used for target verification by qPCR.

GO Term	Gene	RNA Primers	ChIP Primers
cell-cell signaling	Histamine receptor H1(HRH1),	HRH1 5'-TATGGATTATGTGGCCAGCA HRH1 3'-CGGTCATGATCTTGAACCAA	HRH1 5'-GAGCGCATTCACTTCTCAGG HRH1 3'-TGGACCAAGGGACTAGTTGG
	Wingless-related MMTV integration site3 (Wnt3)	Wnt3 5'-TTCTGCCGCAATTACATCGA Wnt3 3'-CCCCTTATGATGTGAGTACA	Wnt3 5'-AATGTCTGCTCCCCGTAGG Wnt3 3'-GAAAGCAAGCCAAAGGAGAA
cell motion & cell motility	Kinesin family member 5C (KIF5C),	KIF5C 5'-GATCCTGAACCTGCTTCTCA KIF5C 3'-TTCGTGCTGTGAGATAAGCA	KIF5C 5'-TCGTACCACGAGGTTCTTC KIF5C 3'-GTGCCAACAGTCTGTGGATG
	Platelet derived growth factor B polypeptide (PDGFB)	PDGFB 5'- ATCTCTCGGAACCTCATCGA PDGFB 3'- ATGTCCCAGGACTTCTAGTCA	PDGFB 5'-TTTCTCTCTTCCCTTTTGC PDGFB 3'-CTGAAGGGTGGCAACTTCTC
synaptic transmission & neurotransmitter transport	Cholinergic receptor, muscarinic 1 (CHRM1)	CHRM1 5'- CAGCTGGAAAAGAAGAGAGGA CHRM1 3'- CTTCTTCTCCTTGACCAGTGA	CHRM1 5'-TGTGAATAATGGGGCATGTG CHRM1 3'-CCTCTCACAAAGGCACACTCT
positive regulation of transcription & positive regulation of gene expression	Short stature homeobox 2 (SHOX2),	SHOX2 5'- CAAAATCAAGCAGAGGGCGAA SHOX2 3'- CTACGTTGACATAGGGTGCA	SHOX2 5'-GCTCTTGCCATTAATCCAG SHOX2 3'-GCAATGCACTTGTGCAATTCC
	Interferon regulatory factor 6(IRF6)	IRF6 5'- CCAAAACAGAACCTCTGGAGA IRF6 3'-ATATGCTCTGGACCTGGGAA	IRF6 5'-GCACTTCATGGAGAATGGAAA IRF6 3'-GTCAATGCACCTTCCAGGAC
regulation of neuron differentiation & Regulation of cell development	Eyes absent 1 homolog (EYA1),	EYA1 5'-AAGCAGCAGCTTAGTCCAA EYA1 3'- CTAAGAAATCCCGTCTGTCCA	EYA1 5'-GTGGGAACCTCTGTCAACAC EYA1 3'- GTATCCGTTCTCCTAAGTTTCAGGT
	Inositol polyphosphate 5-phosphatase J (INPP5J)	INPP5J 5'- CTATCCTTAGCCTCCAGCAA INPP5J 3'- ATTTGTTGGTACCCACGTCA	INPP5J 5'- TGAATCTTCTCCCTTCAAACCTC INPP5J 3'-CTTGACAAGTGCCCTTTCAGAGC

	Gene	RNA Primers
CHRM isoforms (mouse)	Cholinergic receptor, muscarinic 2 (CHRM2)	CHRM2 5'- CCAAAAAGAAGCCTCCTCCA CHRM2 3'- GCGTTACAAAGTGCATAGCA
	Cholinergic receptor, muscarinic 3 (CHRM3)	CHRM3 5'- GAAGAGGATATTGGCTCAGAGA CHRM3 3'- GCTTGGAGAAGTCTTCTGA
	Cholinergic receptor, muscarinic 4 (CHRM4)	CHRM4 5'- GATAACCAGTGCTTCATCCA CHRM4 3'- TAATGCTCGGCTTCATCAGA
	Cholinergic receptor, muscarinic 5 (CHRM5)	CHRM5 5'- GATCTAAGTGCTGACTGGGAA CHRM5 3'- GTAAGTGGGAGTGTCATAGTC A

	Gene	RNA Primers
CHRM isoforms (human)	Cholinergic receptor, muscarinic 1 (CHRM1)	CHRM1 5'- GAGTCCCAAATACAGTCA CHRM1 3'- ATGATGTTGTACGGTGTCCA
	Cholinergic receptor, muscarinic 2 (CHRM2)	CHRM2 5'- ACCCAGGATGAAAACACAGT CHRM2 3'- TTTTTGCAGGCTGCTTAGTC
	Cholinergic receptor, muscarinic 3 (CHRM3)	CHRM3 5'- CAGCTGGCAATTTCTCCTCT CHRM1 3'- CCGATAATCAGATCGGCACA
	Cholinergic receptor, muscarinic 4 (CHRM4)	CHRM4 5'- CTCCGTCATGAACCTTCTCA CHRM4 3'- GACAGGAACTGGATGAAGCA
	Cholinergic receptor, muscarinic 5 (CHRM5)	CHRM5 5'- CTGGCATCATGATTGGCTTG CHRM5 3'- GTTCCCGGTAGATTGACACA

Supplementary Figures

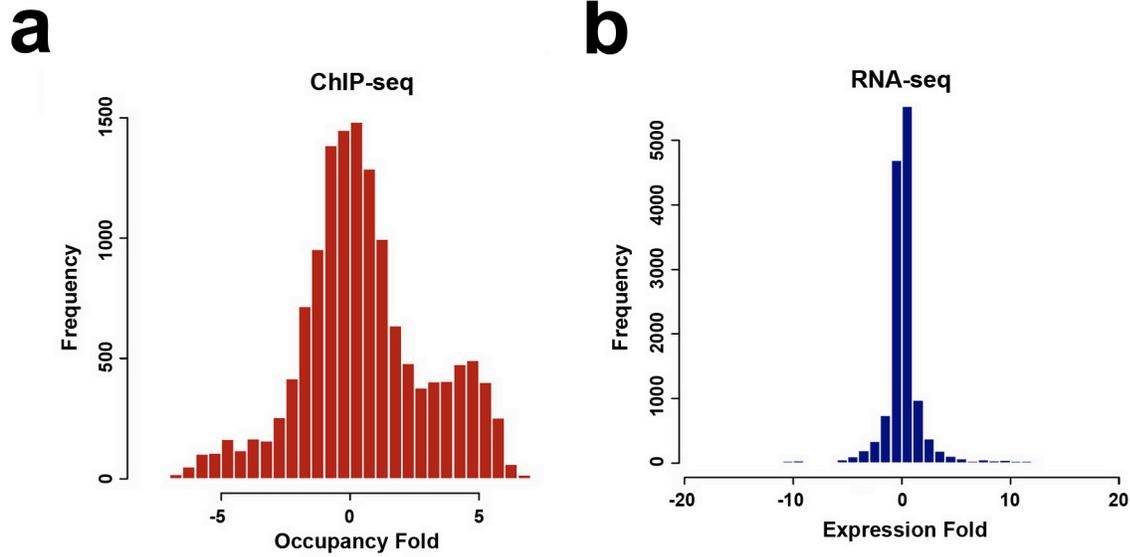


Fig. S1 Fold distribution of H3K9me3 occupancy and RNA expression by genome-wide sequencing. **a** The graph showed that the fold (*STHdhQ111/STHdhQ7*) distribution of H3K9me3 occupancy is increased. **b** The graph exhibited that the fold (*STHdhQ111/STHdhQ7*) distribution of RNA expression follows the normal distribution.

a

	Q7	Q111	total
intergenic	96,940	47,208	144,148
intronic	63,020	32,488	95,508
upstream	3,637	2,244	5,881
downstream	3,160	1,705	4,865
UTR	1,953	1,066	3,019
CDS	2,431	1,339	3,770
total	171,141	86,050	257,191

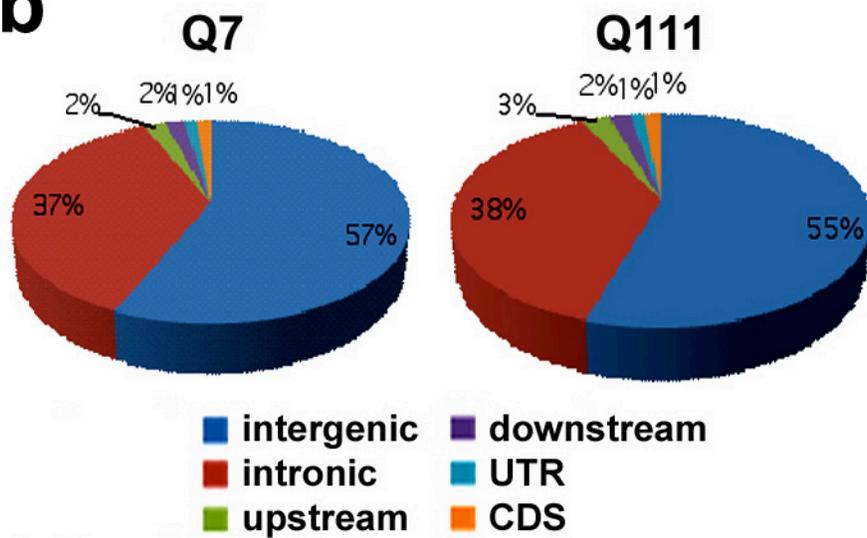
b

Fig. S2 Genomic Structural Occupancy by H3K9me3. **a** The number of H3K9me3 peaks for six different genomic structures in *STHdhQ7/7* (Q7) versus *STHdhQ111/111* (Q111). **b** Pie graphs for the number of peaks in *STHdhQ7/7* versus *STHdhQ111/111*. Pearson's chi-square test for independence: $\chi^2(169.7202)$, p -value $< 2.2e-16$.

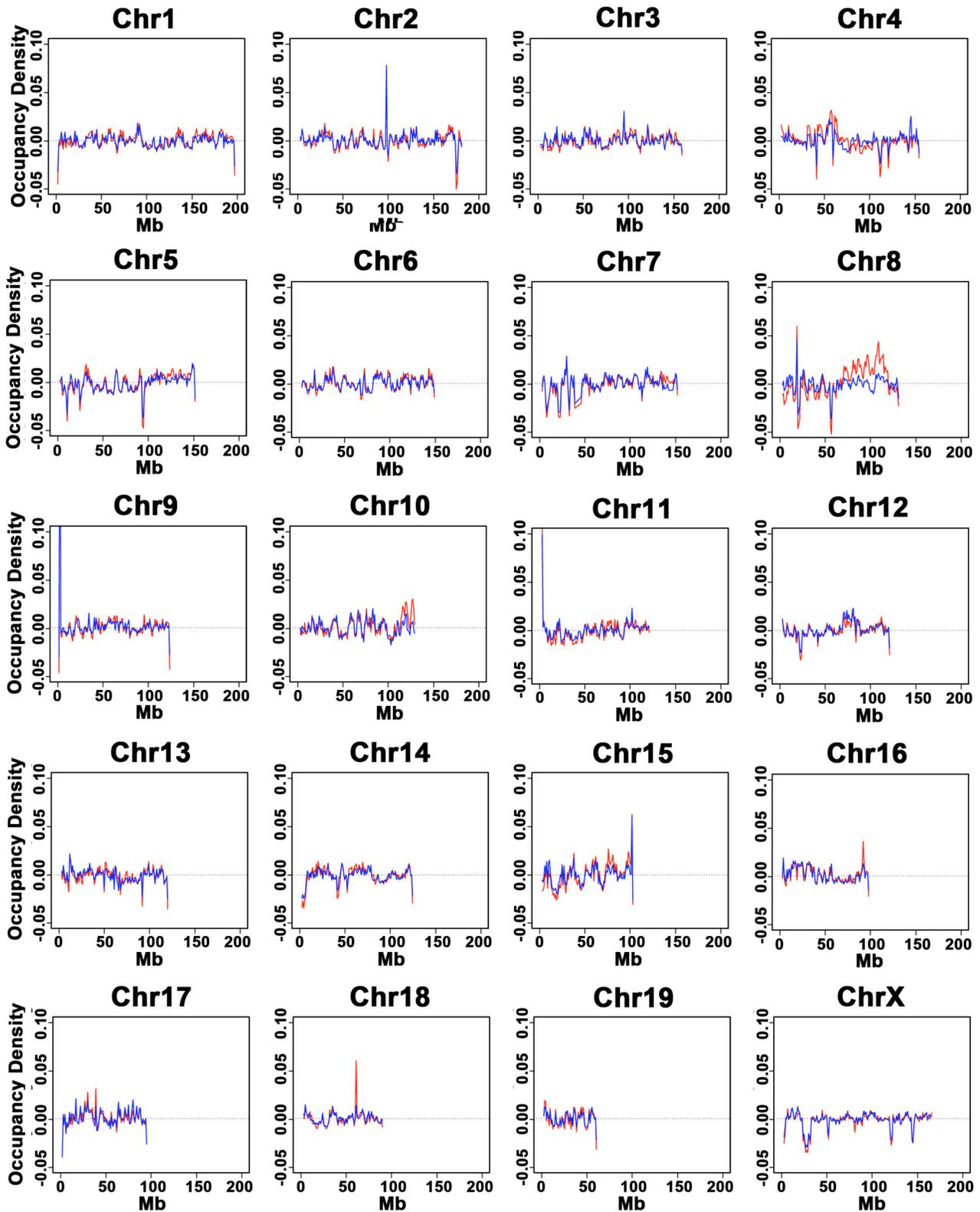


Fig. S3 The chromosomal occupancy of H3K9me3 in *STHdhQ7/7* (blue line) and *STHdhQ111/111* (red line) cells. Coverage density values are shown for H3K9me3 across each chromosome (Chr) as indicated.

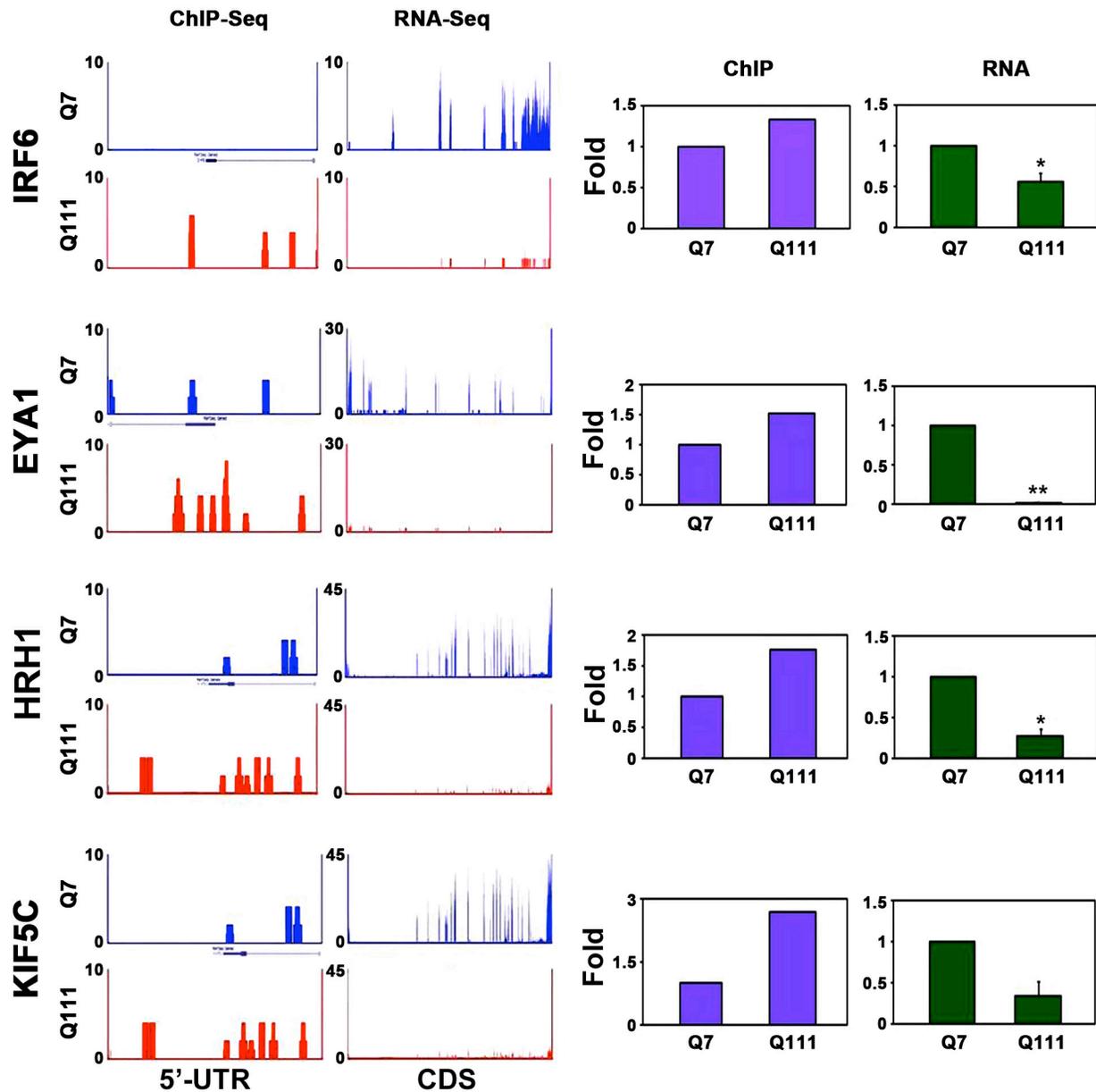


Fig. S4 Verification of H3K9me3-landscaped transcriptome by quantitative real-time PCR. Interferon regulatory factor 6 (IRF6), eyes absent 1 homolog (EYA1), histamine receptor H1 (HRH1), and kinesin family member 5C (KIF5C) is down regulated in *STHdh*Q111/111 (Q111) cells while H3K9me3-occupancy in their promoter is elevated in Q111 cells in comparison to *STHdh*Q7/7 (Q7) cells. Data are mean \pm SE of three separate experiments. Significantly different at $p < 0.05$ (*) and $p < 0.01$ (**).

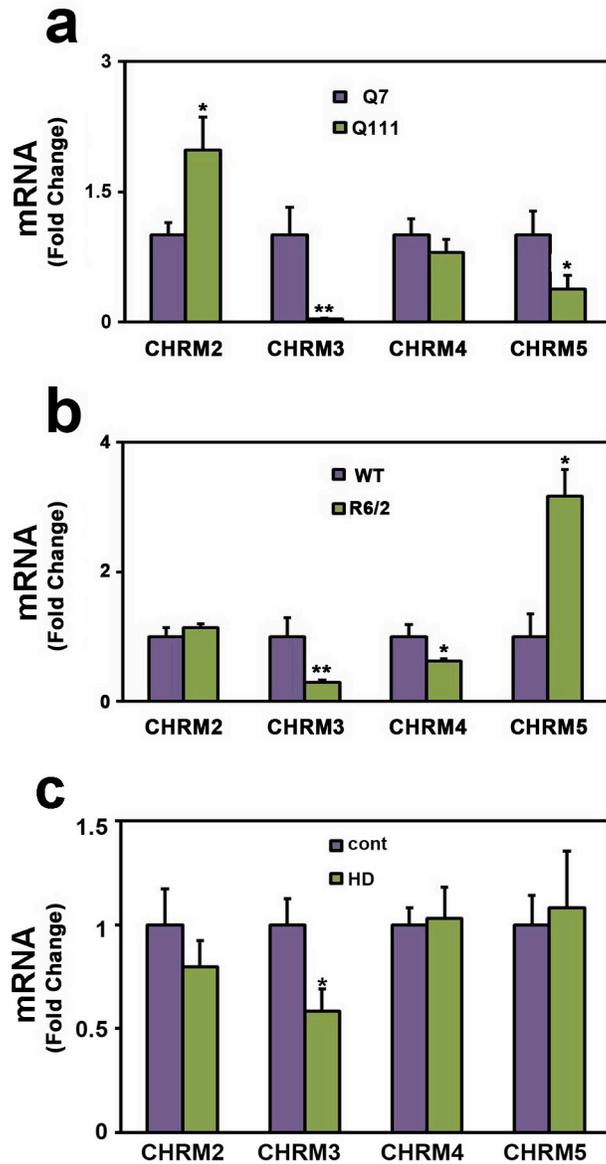


Fig. S5. The differential mRNA expressions of muscarinic cholinergic receptor (CHRM) isoforms in HD. **a** The expression of CHRM subtypes were differentially regulated in *STHdh*Q7/7 (Q7) and *STHdh*Q111/111 (Q111) cells. Data are mean \pm SE of three separate experiments. Significantly different at $p < 0.05$ (*) and $p < 0.01$ (**). **b** The expression of CHRM subtypes were differentially regulated in WT (n=6) and R6/2 mice (n=6). Significantly different at $p < 0.05$ (*) and $p < 0.01$ (**). **c** The expression of CHRM subtypes were differentially regulated in control (Cont) (n=5) and HD patients (n=6). Significantly different at $p < 0.05$ (*).

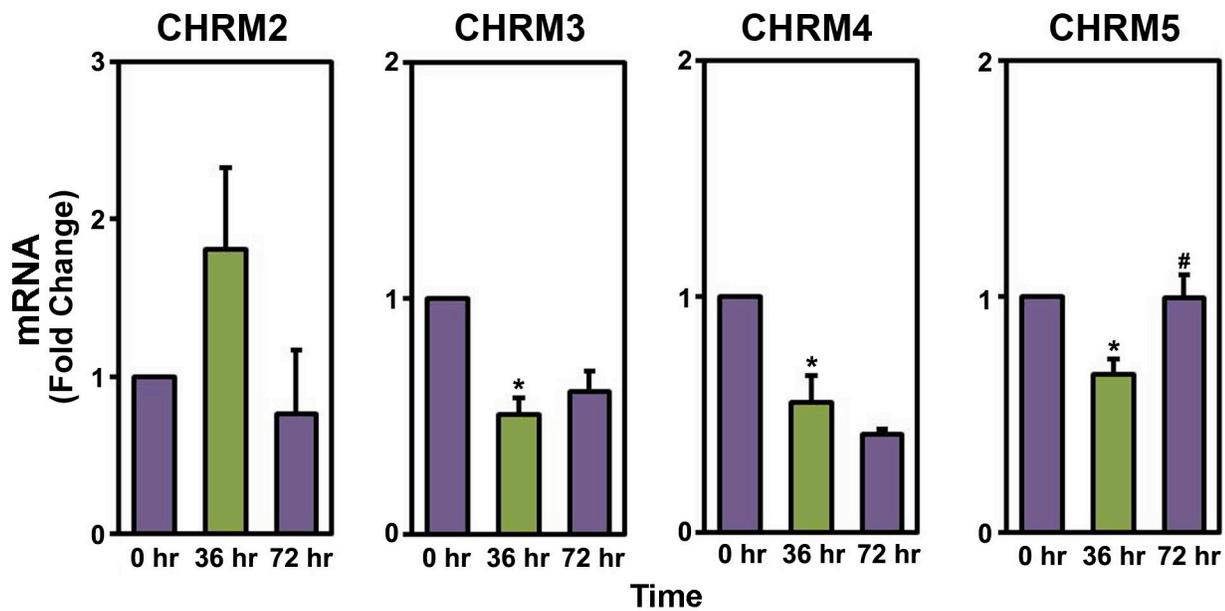


Fig. S6 The modulation of CHRM2-5 mRNA expression by the plasticity of H3K9me3-dependent heterochromatin condensation in Tet-inducible ESET cell line system. Cells were treated with doxycycline (+Doxy) for 36hr to turn on ESET expression. Cells were then washed out doxycycline (-Doxy) and switched to the normal media for another 36hr to turn off ESET expression. Data are mean \pm SE of three separate experiments. **, Significantly different from 0 hr at $p < 0.01$; #, significantly different from 36hr at $p < 0.05$.

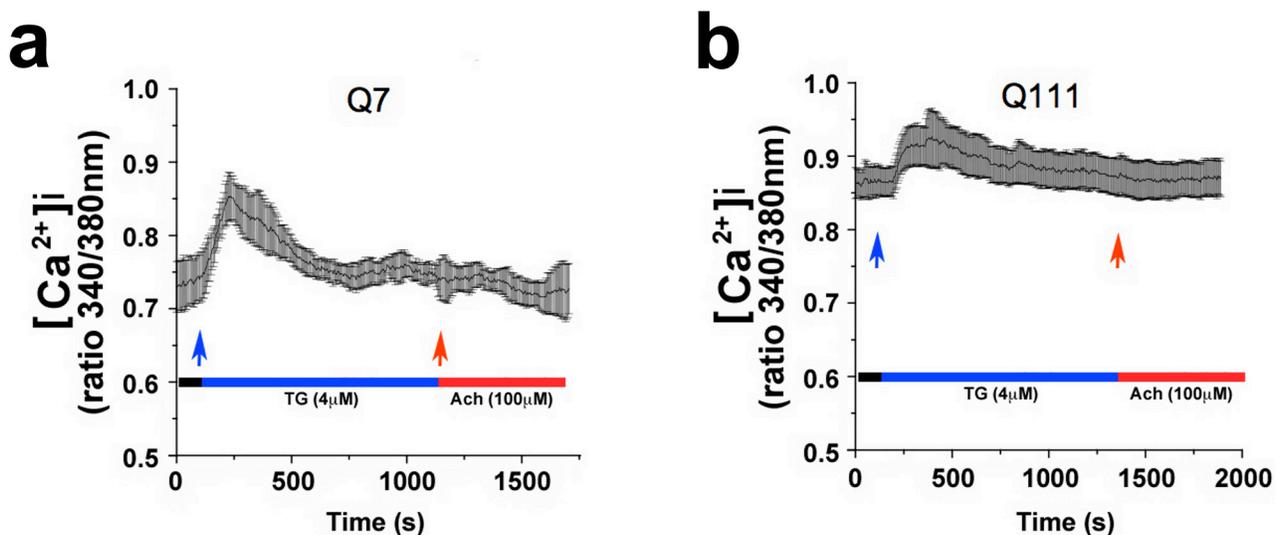


Fig. S7 Acetylcholine (Ach)-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) response is mediated through ER in HD cells. Thapsigargin (TG) induced rapid Ca^{2+} release from intracellular stores by inhibition of the sarcoplasmic/endoplasmic reticulum (ER) Ca^{2+} -dependent ATPase pump without inositol phosphate formation. The basal level of $[Ca^{2+}]_i$ was different between *STHdhQ7/7* (Q7) **a** and *STHdhQ111/111* (Q111) **b** cells. The peak level of $[Ca^{2+}]_i$ was abrogated by TG treatment in both Q7 and Q111 cells.